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Coagulation tests at ambient temperature

Field of invention

According to prior art, laboratory diagnostic coagulation tests are performed at 37°C. This need for a thermostat adds much hardware and costs to the instrumentation and hampers the development toward more point of care (POC) testing. What is needed is more inexpensive and easier to use instruments for coagulation tests. This will be exemplifies with the prothrombin time test (PT).

The PT test is used to diagnose liver disease and coagulation anomalies of the blood and to monitor antithrombotic therapy by vitamin K antagonists. In performing a PT test, sample and thromboplastin reagent are mixed at 37°C and the time needed for the mixture to clot is determined. When the test was first devised more than fifty years ago, this clotting time was identical to the PT, but today, to accommodate variations in test properties, the PT is expressed in International Normalized Ratio (INR), vidi infra.

Most of PT tests are performed on blood plasma at highly automated centralized laboratories. Although this may be economical for generating the test results, it may not be overall economical from the patient's point of view. The reason is the time required to transport samples to the laboratory and the results back. This time can be hours to days, which is long compared to the 10 minutes or less that is required for a PT test to be performed next to the patient, i.e. at the point of care (POC). The transportation time can delay the start of optimal treatment and can force the patient to make additional visits to the physician. In addition, a centralized PT analysis will require a vein puncture, a relatively large blood sample volume and a sturdy blood container. A POC PT analysis would only require finger prick, a small blood volume and no sample contained. Considerations of this kind have prompted the development of POC PT test procedures intended to be performed at doctors' offices, at small laboratories and at patients' homes. The comparison between PT tests performed at centralized laboratories or POC also applies for other coagulation tests. The present invention concerns POC procedures for coagulation tests.

In particular, the present invention focuses on instrument requirements for coagulation testing and on the composition of test kits for this type of analysis.

Background

The following describes PT tests but also applies to other coagulation tests such as activated partial prothrombin time (APTT), whole blood coagulation time and activated coagulation time (ACT).

There are about 6 million patients on vitamin K antagonist therapy who need to have their PT determined about 20 times per year. This makes for 120 million PT tests per year, each of which requires about 15 minutes of health care time and contributes with 10 Euro per test in health care costs, not counting test equipment and consumables which add some 50 million Euro. The health care cost of PT testing in monitoring antithrombotic therapy is thus about 1200 million Euro. If these health care costs could be reduced by 20% by home POC PT testing, while enhance the quality of life for the patient, a health care savings of 240 million Euro would result. Even after an offset by 2 Euro per test in increased cost for equipment and reagents, the savings in the health care sector would be about 200 million Euro.

The result of a PT test is not expressed in clotting time (CT) but rather in International Normalized Ratio (INR). The INR is obtained by comparing the CT for the sample with that of a normal individual, normal clotting time ((NCT). The INR is the ratio between CT and NCT raised to the power of ISI, the International Sensitivity Index. The NCT and ISI are properties of the certain PT test procedure and are obtained by calibrating the test. In the calibration the PT test procedure is direct or indirect aligned with the reference PT test procedure.

Calibration of a PT test involves the whole procedure or method. It involves the reagent(s), the instrument, the volumetric equipment and the instructions for use. Since many users, especially POC PT tests users, want to rely on the calibration of the manufacturer, it follows, that the calibration of a manufacturer will be reliable only if the product delivered, the equipment kit, contains everything needed to perform the PT test. However, according to prior art, this is not the case. Because of the high costs of the instrument, the instrument will be delivered separately and will be used throughout an extended time period, e.g. three to five years. The instrument will not be replaced every time a new lot of reagent is taken into use, which may be about every 6 to 12 months.

The proof of a well functioning PT test is based on results on quality assurance samples. Such samples are known as controls with known INR values. The PT test functions well if the results obtained with the quality assurance sample (the control) must fall within acceptable limits. If it does not, the test is viewed as malfunctioning. Something is wrong, but what? If no user site calibration has been performed, then the reagent, the instrument and volumetric equipment have not been calibrated together. It is then fully possible that every equipment component functions well when tested separately, but that they do not work well together. The user doesn't know where to start in eliminating the analytical problem. The situation would have been much better if all analytical equipment had been delivered as one product; i.e. in one box with one lot number. The manufacturer would then have had the opportunity to calibrate all pieces of equipment together, which would reduces the risk of malfunctions in the first place. And, should malfunctions occur, then there is a natural action to be take – replace the whole equipment kit product and most likely remedy the problem.

For home use of a POC PT test, there are additional advantage in receiving everything, including the instrument, in one equipment kit product. This would allow a patient to have a complete set of test equipment at home, at work and wherever else much time is spent. An inexpensive instrument contained in the PT equipment kit product would bring many advantages to a patient who wants to use a POC PT test procedure.

Obvious there is a need for POC PT test equipment in which the instrument is sufficiently inexpensive to be provided together with a 6 month to 12 month supply of reagents and other consumables. This means that there is a need for a test equipment kit product that contains an instrument together with reagents for 10 to 100 analysis. From this it may be gathered that the manufacturing price of the instrument must be minimal.

It is emphasized, that the above mentioned considerations for PT tests also apply to other, present and future, medical diagnostic coagulation tests. For example, there are new antithrombotic drugs being developed that inhibit coagulation factors Xa and IIa. Treatment with these new drugs will need to be monitored with relevant tests and the need for inexpensive instrumentation for POC test procedures will become evident.

General description of the invention

According to the present invention, an instrument for performing a POC coagulation test, e.g. a POC PT test, can be made sufficiently simple, and therefore sufficiently inexpensive, to allow the instrument to be delivered in the equipment kit product together with reagents. When the reagents and other consumables of the kit product were used up, the instrument could be discarded, or returned to collect some smaller deposit. Such a sufficiently inexpensive instrument is here called a disposable instrument.

A technical difficulty in creating a disposable instrument is the necessity of performing coagulation tests at 37°C. Heating and temperature control are expensive. Apart from hardware for heating and temperature control, the large power consumption requires a power socket connection, which add costs. Therefore, if it were possible to perform a coagulation test without temperature control, i.e. perform the test at ambient temperature, then the possibilities of creating a disposable instrument would be enhanced.

The present inventions thus concerns a procedure to perform a coagulation test at ambient temperature in which the clotting time and the temperature are both measured and used to obtain the test result. The coagulation tests on which the inventioned may be practiced include, but are not limited to, PT, APTT, ACT and spontaneous whole blood coagulation time, and collagen or collagen derivatives activated whole blood coagulation time.

The invention also concerns establishing upper and lower temperature limits outside of which the coagulation test cannot be performed and defining the composition of a test kit product.

Specific description of the invention

The main reason to perform a coagulation tests at ambient temperature is to abolish the need of expensive and energy consuming temperature control equipment. According to the present invention, it possible to perform coagulation tests at the ambient temperatures typical of human dwellings. According to the present invention, it is possible to perform a coagulation test at ambient temperature in which the clotting time and the temperature are measured and used to obtain the test results. In performing a coagulation test according to the invention, there is also a second reason to measure the temperature. This is to qualify the temperature conditions of the test. There will be a temperature range within which the test can be performed and the measured temperature is used to check that the ambient temperature is within this permissible range.

The test results of a PT test is expressed in INR. According to prior art, a PT test is performed at a fixed temperature of 37°C and the INR is a function of the clotting time, INR(CT). According to the invention, a PT test is performed at any temperature within specified limits. The clotting time and the temperature (t) are both measured and the INR is a function of both, INR(CT, t). According to the invention, the temperature is also measured to ascertain that the temperature is within a range where the PT test may be performed.

The coagulation test is intended to be performed at temperatures typical of human dwellings. Hence, the ambient temperature range is assumed to be the indoor temperature range of such a dwellings, i.e. between 15°C and 45°C. It is assumed within the scope of the present invention that the ambient temperature is limited to the range 15°C and 45°C.

A sharp dependency of the clotting time with temperature is likely to reduce the precision of the assay. As evidenced in Example 1, the speed of clot formation is reduced at temperatures below 18°C making clotting less distinct and more difficult to detect. Also at temperatures above 35°C the PT results were dependent on temperature. It is therefore within the scope of the present invention to perform PT test by a procedure in which the ambient temperature is limited to the range of 18°C to 35°C.

In antithrombotic treatment with vitamin K antagonists the optimal blood PT level is in the range of INR 2 to INR 3. The results of experimental work described in Example 1 indicate that there is an optimal temperature range for determination of PT in samples with INR above 2, see Figure 3. This optimal temperature range is about 30°C to 35°C. In performing coagulation tests according to the invention, it may be favorable to limit the ambient temperature to the range of 30°C to 35°C.

Limiting the practice of the invention to a temperature range of 30°C to 35°C may appear to dissipate much of the practical advantages offered by the invention. Yet, it is conceived that it is no major practical complication to have a small volume with temperature controlled between 30°C and 35°C, e.g. a warmed box. Within a doctor's office, a small laboratory or a patient's home such a box could even be seen as an advantage. It may provide a storage facility and a work area. The box could contain proper lighting, for performing the assay. Such a thermostat box will be a none crucial piece of analytical equipment because it is not strictly necessary and because its function is readily checked.

Without departing from the spirit of the invention, other temperature limits than those mentioned above are possible. As indicated, the lower limit of the range of permissible temperatures appears to be the most critical and may needed to be fine tuned, e.g. specified to 22°C. Although, no reason has appeared why the upper limit of permissible temperatures should be lower than 35°C, such reason could well appear. Reduction of the upper limit, e.g. to 30°C, is possible within the scope of the present invention.

As mentioned above, there appears to be an optimal temperature range for determination of PT in samples with INR in the range 2 to 3 or even the range of 2 to 7. In the this optimal temperature range, the temperature dependence of the test result is small which will minimize the impact of bad temperature equilibration and/or determination. In the examples below, this optimal temperature range was about 30°C to 35°C. The important observation is that there is an optimal temperature range and it is conceived that alterations in reagent composition and/or procedural details can shift this optimal temperature range to lower temperatures including those typically found in human dwellings, i.e. 20°C to 30°C. If such a shift in optimal temperature can be accomplished, then there are reasons to adjust the limits of permissible temperature accordingly.

The invention also concerns how to obtain results from PT tests performed according to the invention. In a PT procedure according to the invention, the clotting time and the temperature are determined. As shown in Figure 1 and Figure 2, the NCT and the ISI for a given PT procedure can be viewed as functions of temperature and the nature of these functions may be determined by calibration. The a PT procedure according to the invention can be calibrated by determining the clotting time at various temperatures within the permissible range for samples with known INR. At these temperatures, the NCT and the ISI of the PT procedure are determined and functional relationships between normal NCT and temperature and ISI and temperature. The later are denoted NCT(t) and ISI(t), respectively. Once, NCT(t) and ISI(t) are determied, the INR as function of clotting time (CT) and temperature, INR(CT, t) can be determined as follows:

$$INR(CT, t) = (CT/NCT(t))^{ISI(t)} \qquad \text{Equation 1}$$

In the examples, NCT(t) and ISI(t) were second degree polynomials. Naturally many other types of functions may find use in this context. Any function that gives a good fit to the calibration data may be used..

As alternative to deducing a functional relationship between INR and t and CT, i.e. deducing a two variable expression NCT(t, CT) and computing the assay results using this expression, a table may be constructed. This table will be a matrix where each row is a clotting time (CT) and each column a temperature. Each given pair of temperature and CT will correspond to an INR. Thus, there is no departing from the spirit of the invention if the results of the PT procedure is presented in a table where the results of the test is in position defined by a temperature and a clotting time.

Without departing from the scope of the invention, any method that allows an INR value to be deduced from a CT and a temperature may be employed. The same is true for assay conditions. These may include any limited permissible temperature range and any limited permissible clotting time range. In the experimental work there are temperature ranges identified for which the temperature dependence is negligable and which makes the INR value a function of the clotting time alone. Also in this case the PT test is according to the invention. The clotting time and the temperature are both measured and used to obtain the test results. The temperature is measured to ascertain that the temperature is within a permissible range.

It is a part of the invention to provide users equipment needed to perform a PT procedure according to the invention. The equipment is provided as a test kit which contains reagents for 10 to 100 PT analysis, an instrument and instructions for use.

Examples

The following examples are given to a better understanding of the invention and to show that the invention is of practical and economic importance. The examples show but a trifle of all possible embodiments of the invention. Therefore, the examples do not limit in any way the scope and sprit of the present invention.

Materials and Methods.

PT reagent was GHI 131 lot C225F which is of the Owren's kind in that it contains rabbit brain thromboplastin and bovine factor V and bovine fibrinogen. The reagent, supplied as a lyophilized powder. One vial of reagent was reconstituted in first 5 mL of water and then 5 mL 25 mM $CaCl_2$. The reagent was used within 4 to 10 hours of reconstitution.

Control plasma samples with known INR were lyophilized plasma samples with known INR. One control plasma, product number GHI 162, had an INR of 1,14 and the other, product number GHI 167, had an INR of 2,82. The lyophilized control plasmas were first reconstituted in 1 mL of water according to the manufacturers recommendation, and then, to give the reconstituted control plasma about the same PT potency as blood with erythrocyte fraction volume (EVF) of 0.45, the control plasma were diluted by addition of 0.82 mL of 150 mM NaCl. PT reagent and control plasma were manufactured by Global Hemostasis Institutet MGR AB, Linköping, Sweden.

Citrated plasma samples from the Department of Clinical Chemistry, University Hospital, Linköping, Sweden. The 23 samples were from those arriving to the department for analysis of PT during about 3 hours in the afternoon of June 12, 2003. The samples were analyzed according to the invention within 4 hours of being analyzed by the routine procedure of the department. The samples were made untraceable to preserve the anonymity of the patients and were provided with the PT values obtained by the department. Prior to assay according to the invention, a 200 µL portion of each plasma was diluted 1:1.82 by addition of 164 µL of 150 mM NaCl. This was to make the equipotent with blood with EVF of 0,45. The experiments were approved by the local ethical committee of the Medical Faculty of the University of Linköping.

Glass capillaries with a volume of 25 µL (length 50 mm), product number 111.395-25, were from KEBO-lab, Stockholm., Sweden.

The PT reagent was added to 8 mm inner diameter polystyrene tubes, 300 µL of reagent in each. The tubes were capped and temperature equilibrated in a water bath. At time zero, 25 µL of sample was added to a reagent tube and recapped. The tube was tilted once about every second so that the bottom of the tube was out of water bath when tilted and back in the water bath when straight up. The contents was inspected every time the tube was tilted. When first signs of clotting were detected the time was recorded. The temperature in the water bath was

varied between 14°C and 40°C. The 25 µL of sample was added either by glass capillary or by pipette with disposable plastic tip.

Example 1.

With a PT procedure, the clotting time (CT) was determined for two control plasma samples at every two degrees centigrade in the temperature range of 18°C to 38°C. The control plasma samples had INR values of 1.14 and 2.82. From these values, the Normal Clotting Time (NCT) and International Sensitivity Index (ISI) of the PT procedure were calculated at each of the eleven temperatures. The NCT was in the range of 55 s to 22 s and the ISI was in the range 1.0 to 1.5, SEE Figures 1 and 2. Second degree polynomials were fitted by minimal least square method to give NCT and ISI as functions of temperature. The second degree polynomials that gave the best fit to the data were:

$$NCT(t) = 136.4 - 6.165t + 0.0837t^2$$

$$ISI(t) = 1.0584 + 0.0420t - 0.0011t^2$$

NCT and ISI for the same PT procedue was also determned at three temperatures, 18.5°C, 22.7°C and 28.4°C with patient plasma samples from the Department of Clinical Chemistry. Figure 1 and 2 also show that the estimates of NCT and ISI made with the patient plasma samples and the INR values supplied by the department were well in line.

The functional relationship between the temperature and NCT and ISI allowed the construction of schools of curves, each for a specific INR value, showing the expected clotting time as function of temperature. The curve for INR 1 flattened out in the temperature range of 35°C to 40°C. The curves for higher INR values displayed an increasingly pronounced minimum in the range 30°C to 35°C.

Example 1 demonstrates that functional relationships temperature and NCT and ISI, NCT(t) and ISI(t), can be determined. Furthermore, Example 1 demonstrates that every value pair (t, CT) translates into an INR value. Figure 3 could be used for such a translation but an interpolation between the curves is necessary. A two variable mathematical relationship or a table appears to be the most straight forward means to translate the (t, CT) value pair into an INR value. Figure 3 clearly demonstrates that a translation of the kind can be performed in practice.

When a sample is analyzed according to the invention, the clotting time and the ambient temperature (t) are measured. With these values and known functional relationships NCT (t) and ISI(t), an INR value can be computed as $INR = (CT/NCT(t))^{ISI(t)}$. Thus, according to the invention, provided the temperature is within a permissible range, every value pair (t, CT) will generate an INR value. Naturally, the CT must also be within some permissible range but this does not represent anything new in comparison to state of art PT procedures and is therefore not elaborated on here.

Example 2.

Plasma samples from 23 patients were obtained from the Department of Clinical Chemistry together with INR values determined according to prior art. The 23 samples were analyzed according to the invention. The ambient temperature and the clotting time for each sample was measured. The temperature in the series was stable at 22.7°C. At this temperature, the NCT and ISI of the PT procedure was determined with respect to the prior art INR values. INR values according to the invention were then computed. The INR values obtained according to the invention at 22.7°C were compared to the INR values obtained according to prior art at 37°C, see Figure 4. The comparison was by linear regression. A slope of near unity (1.008), an intercept close to zero (0.007) and a correlation coefficient was 0.982 was obtained. There were no obvious outliers. The first 12 of the 23 samples were similarly analyzed at 18.5°C and 28.4°C. For each of these 12 sample a mean INR value was determined as the average of three determinations, one at each of the three mentioned temperatures. This mean INR value was also compared by linear regression analysis to the INR values supplied by the department, Figure 5. Interestingly, the mean INR values correlate more strongly with the INR values supplied by the department than the INR values obtained at only one temperature (22.7°C), squared regression coefficient was 0.995 compared to 0.982. Inspection of the data in Figure 5, shows no bias and no obvious outliers. It appears that INR can be determined according to the invention at any temperature in the range of 18°C to 40°C.

Example 2 demonstrates that INR values can be determined at other temperatures that 37°C. According to the data, a PT test can be performed at ambient temperature provided that the clotting time and the temperature are measured and that both of these values are used to derive the test result. To further prove the point, mean INR values were determined for 12 patient plasma samples. Each mean INR value was an aritmetric average of three INR determinations, one at 18.5°C, a second at 22.7°C and a third at 28.4°C. The mean INR values and the ptior art INR values were compared in Figure 5. In this case the data lined up even better that in Figure 4. The results are interpreted in the following way; each mean INR value is the average three determinations according to the invention. Each of these three estimates is equally good, regardless of temperature. The mean INR values are therefore better estimates of the true INR value than the single estimate at 22.7°C. A standard deviation of the determinations are obtained by assuming that the INR value of the deparument has correct. The standard deviation for the mean INR and the 22.7°C INR can then be determined to 0,029 and 0.043, respectively. The ratio between these standard deviations is 1.5 which is close to 1.73, the square root of three, which is expected if all determinations, regardless of temperature, carry the same weight.

The data shown in Figures 4 and 5 displays a discovery on which the invention is based. PT can be determined at any temperature. The temperature needs only be measured and used together with the clotting time to obtain the assay result. The fact that PT can be determined over a relatively broad temperature range is not trivial. It is a fact that now has been experimentally verified. Now we know, that new analytical possibilities, offering considerable advantages, open themselves unto us. Since the temperature does not by necessity need to be defined, it only needs to be measured, much expensive hardware can be stripped from the instrument used for PT determination. It could well be that the instruments can perhaps be made so inexpensive that a disposable instrument for PT tests is possible. For POC PT testing this could be decisive.

PT determination according to the invention could result in improved test quality. One reason for a bad PT result is temperature fault. The temperature in the reaction mixture is not what it is assumed to be. This could be because of the temperature has not had the time to equilibrate, or because of some error in the thermostat. It stands clear to reason, it is easier to measure a temperature with good precision than to keep it at some predetermined level with the same good precision.

Table 1. The PT clotting time for the two control plasma samples were determined at various temperatures in the range of 18°C to 38°C. The clotting times are designated CT INR 1.14 and CT INR 2.82. Using these two CT values, the NCT and the ISI of the PT procedure was determined at each temperature from the relationship $INR = (CT/NCT)^{ISI}$. All clotting times are given in seconds.

Temperature	CT INR 1.14	CT INR 2.82	NCT	ISI
18°C	57	109	51.9	1.40
20°C	52	97	47.5	1.45
22°C	45	83	41.2	1.48
24°C	40	75	36.5	1.44
26°C	35	68	31.8	1.36
28°C	33	63	30.1	1.40
30°C	30	61	27.1	1.28
32°C	27	60	24.1	1.13
34°C	26	57	23.2	1.15
36°C	26	57	23.2	1.15
38°C	26	63	22.9	1.02

Table 2. Below is a piece of an Excel file showing that a clotting time and a temperature, measured when practicing a PT procedure according to the invention, can be translated into an INR value. There is one row for each CT in seconds and one column for each temperature in degrees centigrade. If in practicing a PT procedure according to the invention, a temperature of 24°C and a clotting time of 45 seconds are measured, then the INR of the sample is 1.34. The example is from a part of the world where a comma, instead of a decimal point is used. The section of a matrix shown covers the temperature range 19°C to 28°C. If, in connection with a PT test performed according to the invention, a temperature of 18°C is determined, then this outside the conditions under which the test is intended, and no INR value is obtained. The temperature measured is thus used to qualify test conditions. If the measured temperature is outside the permissible range, then the test conditions are not qualified and no test result will be generated.

	Temperature (°C)									
	19	20	21	22	23	24	25	26	27	28
CT: 29	0,46	0,50	0,55	0,60	0,66	0,71	0,78	0,84	0,91	0,98
30	0,48	0,53	0,58	0,63	0,69	0,75	0,82	0,89	0,96	1,03
31	0,51	0,55	0,60	0,66	0,72	0,79	0,86	0,93	1,00	1,08
32	0,53	0,58	0,63	0,69	0,76	0,82	0,90	0,97	1,05	1,12
33	0,55	0,60	0,66	0,72	0,79	0,86	0,94	1,01	1,09	1,17
34	0,58	0,63	0,69	0,75	0,82	0,90	0,98	1,06	1,14	1,22
35	0,60	0,66	0,72	0,79	0,86	0,94	1,02	1,10	1,19	1,27
36	0,63	0,69	0,75	0,82	0,89	0,97	1,06	1,15	1,23	1,32
37	0,65	0,71	0,78	0,85	0,93	1,01	1,10	1,19	1,28	1,37
38	0,68	0,74	0,81	0,89	0,97	1,05	1,14	1,24	1,33	1,42
39	0,71	0,77	0,84	0,92	1,00	1,09	1,19	1,28	1,38	1,47
40	0,73	0,80	0,87	0,96	1,04	1,13	1,23	1,33	1,43	1,53
41	0,76	0,83	0,91	0,99	1,08	1,17	1,27	1,38	1,48	1,58
42	0,79	0,86	0,94	1,03	1,12	1,22	1,32	1,42	1,53	1,63
43	0,81	0,89	0,97	1,06	1,16	1,26	1,36	1,47	1,58	1,68
44	0,84	0,92	1,01	1,10	1,20	1,30	1,41	1,52	1,63	1,74
45	0,87	0,95	1,04	1,13	1,23	1,34	1,45	1,57	1,68	1,79
46	0,90	0,98	1,07	1,17	1,27	1,38	1,50	1,62	1,73	1,85
47	0,93	1,01	1,11	1,21	1,31	1,43	1,55	1,67	1,79	1,90
48	0,96	1,04	1,14	1,24	1,36	1,47	1,59	1,72	1,84	1,96
49	0,99	1,08	1,18	1,28	1,40	1,52	1,64	1,77	1,89	2,02
50	1,02	1,11	1,21	1,32	1,44	1,56	1,69	1,82	1,95	2,07

Legends to the figures

Figure 1. The filled circles show the NCT of Table 1. The solid line is second degree polynomial fitted to the experimental data points by minimal least square method. The open circles show the NCT values for the PT procedure estimated with the patient plasma samples and the INR values for these supplied by the department. The fitted function was $NCT(t) = 136.4 - 6.165t + 0.0837t^2$

Figure 2. The filled circles show the experimentally determined ISI data of Table 1. The solid line is second degree polynomial fitted to the experimental data points by minimal least square method. The open circles show the NCT values for the PT procedure estimated with the patient plasma samples and the INR values for these supplied by the department. The fitted function was $ISI(t) = 1.0584 + 0.04201t - 0.0011t^2$.

Figure 3. The solid lines show the expected clotting time with the PT procedure for samples with INR values of 1, 2, 3, 4, 5 and 7 in the temperature range of 18°C to 40°C. The calculations were made with the relationship $INR = (CT/NCT)^{ISI}$, rearranged into $INR = (CT/NCT(t))^{ISI(t)}$, with insertions of the NCT(t) and ISI(t) given in legends to Figures 1 and 2.

Figure 4. INR values of 23 patient citrated plasma samples determined according to the invention at 22.7°C are compared with INR values for the same samples determined according to prior art. by the Department of Clinical Chemistry at the University Hospital, Linköping, Sweden.

Figure 5. For 12 patient plasma samples the average INR value of three determinations according to the invention, at 18.5°C, 22.7°C and 28.4°C, were compared to the INR values of the same samples determined according to prior art. The determinations according to the invention and according to prior art are denoted INR invention and INR dept clin chem, respectively.

Claims

1. A procedure to perform a coagulation test at ambient temperature in which the clotting time and the temperature are both measured and used to obtain the test results.

2. A procedure according to claim 1 in which the coagulation test is prothrombin time (PT) and the test result is expressed in International Normalized Ratio (INR).

3. A procedure according to claim 1 in which the coagulation test contained within the group defined by activated partial prothrombin time (APTT), spontaneous whole blood coagulation time, activated coagulation time (ACT) and collagen activated whole blood coagulation time

4. A procedure according to claim 1 through 3 in which the measured temperature is also used to qualify temperature conditions of the test.

5. A procedure according to any of claims 1 through 3 in which the ambient temperature is limited to the range of 15°C to 45°C.

6. A procedure according to claim 1 through 3 in which the ambient temperature is limited to the range of 18°C to 35°C.

7. A procedure according to claims 1 and 2 in which the ambient temperature is limited to the range of 30°C to 35°C.

8. A procedure according to claim 1 through 6 in which the International Sensitivity Index (ISI) and Normal Clotting Time (NCT) are expressed as functions of temperature, ISI(t) and NCT(t), respectively, and the International Normalized Ratio (INR) of the sample is calculated from the clotting time (CT) of the sample and the temperature according to $INR = (CT/(NCT(t))^{ISI(t)}$.

9. A procedure according to claim 1 through 6 where the test result is obtained from a table with rows and columns where one is for various clotting times and the other various temperatures and the test result is found in the intersection of clotting time and temperature.

10. A test kit for performing a coagulation test according to any of the above claims which contains reagents for 10 to 100 analysis, an instrument and an instructions for use.

Coagulation tests at ambient temperature

Abstract

Development of point of care (POC) coagulation tests is hampered by complicated instrumentation, as exemplified by prothrombin time (PT) testing The health care cost of monitoring the about 6 million patients treated with vitamin K antagonists is about 1200 million Euro. These costs could be much reduced if more patients did their own testing. This requires instruments that are more inexpensive and easier to use. The prior art requirement of performing tests at 37°C adds much expensive hardware and complicates testing. According to the invention, coagulation tests can be performed at ambient temperature by measuring both clotting time and temperature and using both to obtain the result. The invention includes methods for treating the data and teaches the use of the measured temperature to qualify test conditions. It defines temperature limits within which the test can be performed and the composition of commercial products for practicing the invention.

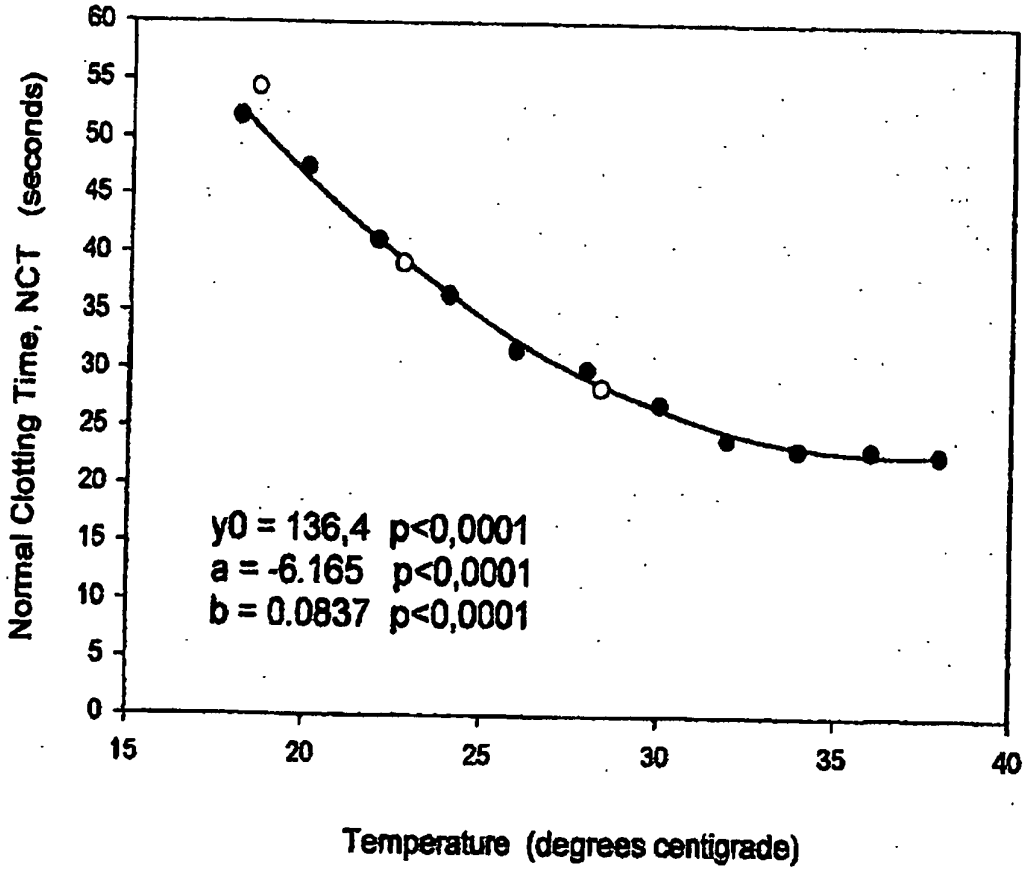

Figure 1

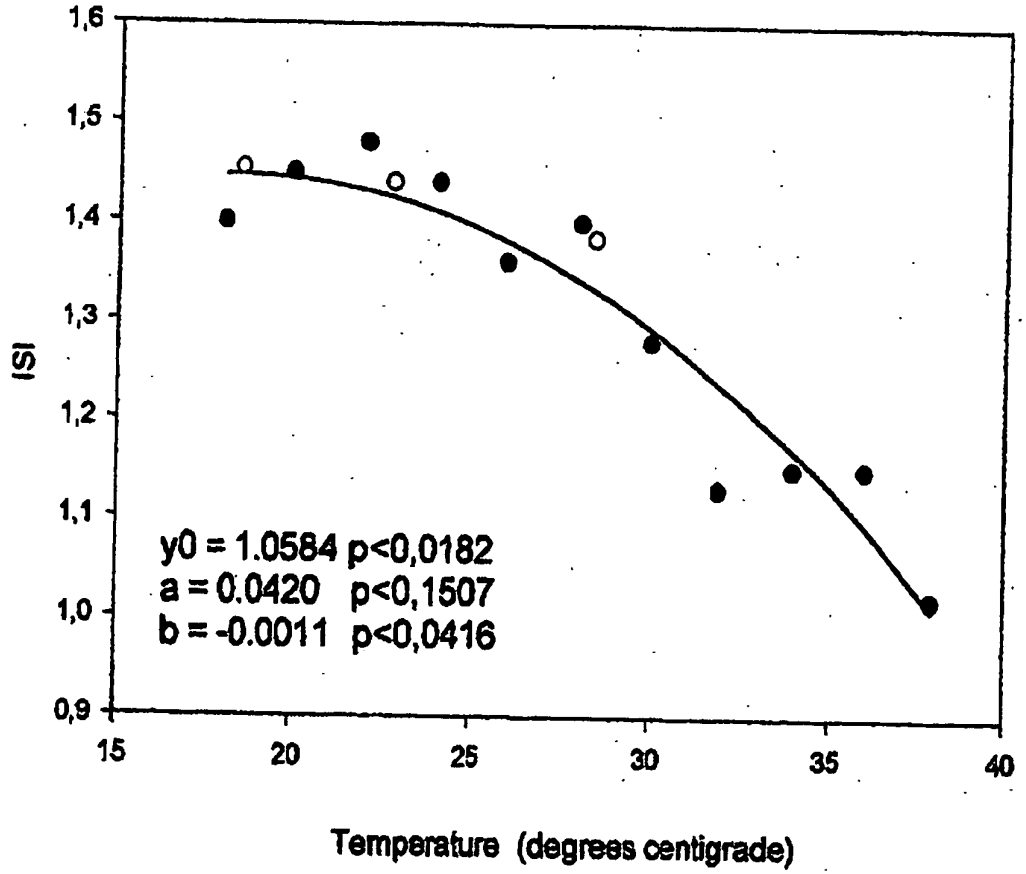

Figure 2

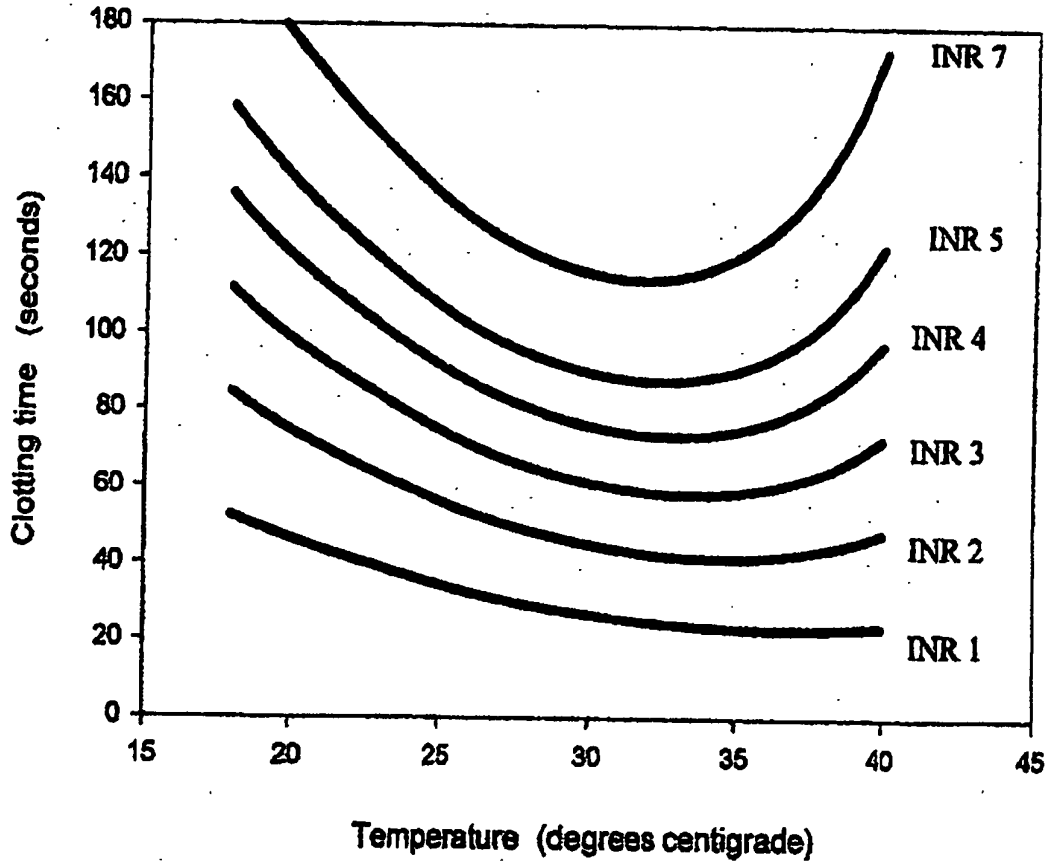

Figure 3

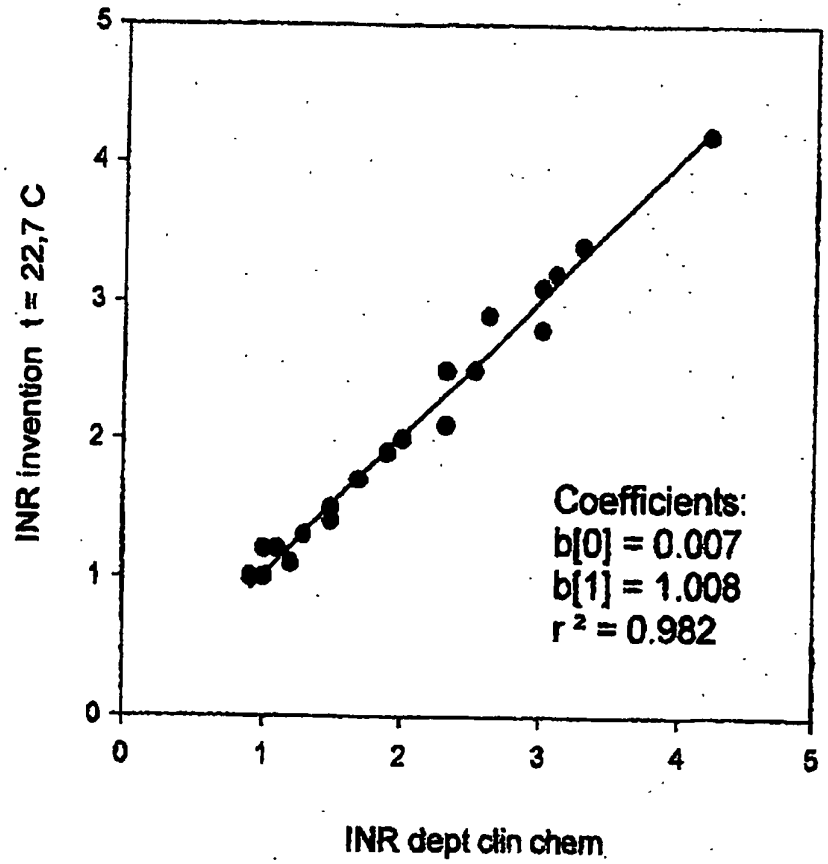

Figure 4

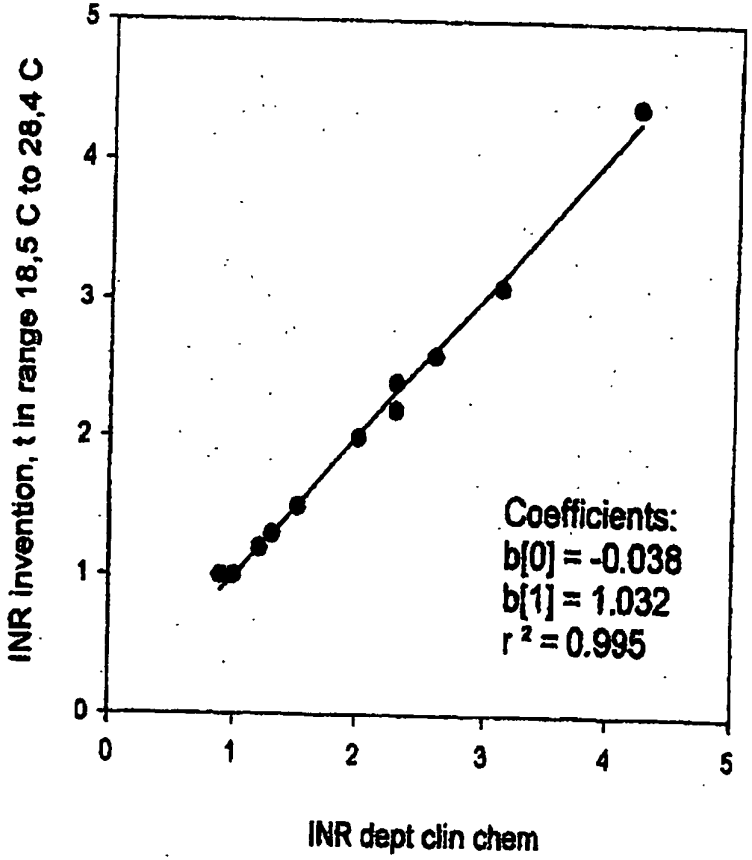

Figure 5